

# Proposed Note to "Nature".

## CONCERNING THE USE OF THE TERM PROTOPLAST

Treatment of Bacillus megaterium in certain media with lysozyme converts the rod-shaped cells into spherical units which are very sensitive to osmotic shock<sup>1</sup>. These structures have been called PROTOPLASTS and it has been established that the cell wall as identified microscopically, chemically, immunochemically, and by its reaction with bacteriophage, is entirely removed by the action of lysozyme<sup>2,3,4</sup>. Similar, osmotically-sensitive, spherical forms can be prepared from other Gram-positive species by various techniques<sup>5</sup>. There is accumulating evidence that the cell wall is absent from such forms although the whole of the cytoplasm is present bounded by an osmotic barrier<sup>2a,b</sup> and capable of most of the activities of the intact cell<sup>2c</sup>.

It is useful to have the term PROTOPLAST to describe such a structure and the phrase PROTOPLAST MEMBRANE to denote the envelope which surrounds it and which is almost certainly identical with the cytoplasmic membrane or plasma membrane of the intact cell. The existing term CYTOPLASMIC MEMBRANE is to be preferred if this identity is subsequently proven.

During the past year methods have been described for preparing "protoplasts" of Gram-negative bacteria such as Escherichia coli and a number of papers have appeared dealing with their physiological and biochemical properties. In a single issue of "Nature" recently there appeared an article on "Protoplasts of E. coli as sources and acceptors of deoxypentose nucleic acid"<sup>6</sup> and a letter on the "Growth and properties of bacterial protoplasts"<sup>7</sup>. In neither case was evidence presented that the cell walls were entirely absent.

The cell walls of both Gram-positive and Gram-negative bacteria (including E. coli) can be isolated by similar techniques but those of the latter are more complex<sup>2d,8</sup>. They contain lipoprotein and lipopolysaccharide as well as mucopolysaccharide which is the only component of many types of Gram-positive cell wall. Five methods have been used to convert E. coli and other Gram-negative bacteria into osmotically-sensitive, spheres - lysozyme treatment at pH 9.0<sup>9</sup> or in conjunction with chelating agents<sup>10</sup>, bacteriophage action<sup>11</sup>, growth in the presence of penicillin<sup>12,13</sup>, deprivation of diaminopimelic acid<sup>15</sup>, growth in media containing 1 - 5% glycine<sup>14,7</sup>. The first four of these methods are thought to involve a mucopolysaccharide component and there is as yet no evidence that other cell wall constituents are removed or are prevented from being incorporated. Indeed, the major component (lipoprotein comprises 80% of the wall of E. coli and may be the outermost layer<sup>15</sup>) may very possibly still be present in spherical forms. Nevertheless, these structures are being referred to as protoplasts and frequently it is explicitly stated that they are "deprived of their cell walls"<sup>6</sup> or "lost their cell walls in a few hours"<sup>7</sup>. This may be so but adequate evidence has not yet been reported.

In an attempt to avoid a situation in which the word PROTOPLAST in one context means that part of a bacterial cell which lies within the cell wall, and in another context means something different, we suggest that it be restricted to the former i.e. it be used to describe a structure in which the cell wall is known to be absent, or alternatively, for that part of the cell which lies within the cell wall and which in some species can be plasmolysed away from it (this is also the general botanical usage<sup>16</sup> - the term was probably first used in 1880 by Hanstein<sup>16a</sup>).

If this definition is accepted, there are certain properties which isolated protoplasts will exhibit and certain tests which can be applied to determine whether or not a particular preparation consists of protoplasts.

a) Morphology and osmotic sensitivity - the internal osmotic pressure of a bacterial cell is normally resisted by the cell wall<sup>2a,b</sup>. If the wall is removed, the protoplast will lyse unless a medium of suitable composition is present to balance this pressure<sup>12d</sup>. In a suitable medium, the resulting form is usually a sphere irrespective of the shape of the cell from which it comes<sup>2a,c</sup>. Electron microscopy of thin sections can indicate the absence of the cell wall<sup>17</sup>.

b) Immunochemistry - cell walls contain specific antigens which are absent from the protoplasts<sup>3</sup>. There will not be cross reactions between protoplasts and cell wall anti-sera.

c) Chemistry - substances which occur exclusively as cell wall constituents will be absent from protoplasts or present only as transient intermediates in the soluble fraction of the cytoplasm. In this category is diaminopimelic acid<sup>2c,12d</sup> and probably certain D-amino acids<sup>18</sup> and sugars<sup>19</sup>.

d) Interaction with bacteriophage - bacterial viruses adsorb by a highly specific reaction between the phage tail and receptor sites in the bacterial cell wall. These sites are absent from protoplasts<sup>20</sup>.

e) Behaviour of isolated cell walls - if enzymic digestion of isolated walls results in their complete dissolution<sup>4,5c</sup>, then protoplasts can be expected to result from the action of the enzyme on whole cells. If digestion of walls releases minor components only, leaving a well-organised residual structure, then it is unlikely that treatment of whole cells with the enzyme will yield true protoplasts.

It may be significant of a difference in structure that whereas protoplasts of Bacillus megaterium have never been reported to be able to grow new walls, to revert to bacillary form, or to give rise to colonies on solid media<sup>2c</sup>, it is often said of E. coli that the "spherical protoplasts had reverted to rods" <sup>6</sup>. There is indeed increasing evidence of the presence of cell wall components in globular forms of E. coli<sup>21</sup> and P. vulgaris<sup>22</sup> and the relationship of these to L-forms is being actively investigated. We recognise the need for a general term to describe bacterial forms which have impaired cell walls, including those in which the wall is absent, but we suggest that until it is established whether or not all the cell wall components are absent from a SPHERICAL FORM or GLOBULAR FORM, the term PROTOPLAST should be avoided.

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